

# OVARIAN FOLLICULAR GROWTH, FUNCTION AND TURNOVER IN CATTLE: A REVIEW<sup>1</sup>

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## ABSTRACT

Studies in cattle assessing changes in number and size of antral follicles, concentrations of estradiol, androgens and progesterone in serum and follicular fluid, and numbers of gonadotropin receptors per follicle during repetitive estrous cycles and postpartum anestrus are reviewed. The rate of growth of small follicles (1 to 3 mm) into larger follicles increases as the estrous cycle progresses from d 1 to 18 (d 0 = estrus). Size of the largest antral follicle present on the ovary also increases with advancement of the estrous cycle. Most large follicles (>10 mm) persist on the ovarian surface for 5 d or more between d 3 and 13 of the bovine estrous cycle. After d 13, most of these large follicles are replaced more frequently by new growing follicles (turnover) with an increased probability for recruitment of the ovulatory follicle after d 18. More research is needed to determine the time required for growth of bovine follicles from small to large antral size and evoke recruitment of the ovulatory follicle. Factors that regulate selection of the ovulatory follicle are unknown but may involve increased frequency of LH pulses in blood, altered blood flow and/or changes in intrafollicular steroids and proteins. Quantitative evaluation of ovarian follicles indicated occurrence of consistent short-term changes in fluid estradiol and numbers of luteinizing hormone receptors in cells of large follicles only during the pre-ovulatory period. Presumably, low concentrations of follicular estradiol found during most of the estrous cycle are not due to a lack of aromatizable precursor or follicle-stimulating hormone receptors. Follicular fluid concentrations of progesterone increase only near the time of ovulation. Little is known about changes in follicular growth, turnover and function during postpartum anestrus in cattle. However, preliminary data suggest that the steroidogenic capacity of large follicles changes markedly during the postpartum period.

(Key Words: Ovaries, Steroidogenesis, Gonadotropins, Receptors, Postpartum Interval, Cattle.)

## Introduction

Ovarian follicular development in cattle during the estrous cycle, at present, is not well understood. Although several investigators have suggested that follicular development is continuous and independent from the phases of the cycle (Choudary et al., 1968; Donaldson and Hansel, 1968; Lobel and Levy, 1968; Marion and Gier, 1968, 1971; Dufour et al., 1972), the notion has persisted that only two

waves of follicular growth exist during the cycle, an idea Rajakoski introduced in 1960 (Rajakoski, 1960). Unfortunately, Rajakoski's conclusions were based upon a qualitative assessment of data without current knowledge of the profiles of gonadotropins and of ovarian steroids in blood during the bovine estrous cycle. A better understanding of bovine folliculogenesis is essential to deal with problems such as variable responses to superovulation and estrous synchronization treatments (Anderson, 1978; Hasler et al., 1983; Monniaux et al., 1983). Therefore, this review addresses follicular growth and function in cattle, not only during repetitive estrous cycles, but also during postpartum anestrus and prepubertal growth, utilizing current developments.

*Definition of Folliculogenesis.* Folliculogenesis may be defined as formation of Graafian (mature, preovulatory) follicles from a pool of primordial (non-growing) follicles. The pool of primordial follicles remains stable

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(average = 133,000 follicles from birth until about the fourth year of life, but numbers of them subsequently decline until approximately 3,000 remain in ovaries of cows 15 to 20 yr old (Erickson, 1966). Erickson (1966) separated growing follicles into two groups: preantral and antral. Both types of growing follicles increase coincidentally in number from birth to about 70 d of age (Erickson, 1966; Goff et al., 1984). Erickson (1966) observed that numbers of growing preantral follicles remain constant (200 to 250 per pair of ovaries) in cows until 4 yr of age, and then decline coincident with the decrease in primordial follicles until fewer than 50% of maximal numbers remain in cows 15 to 20 yr of age. In contrast, antral follicle numbers remain constant (30 to 60 per pair of ovaries) in cows to 10 yr of age and then decline to less than 50% of maximal numbers at 15 to 20 yr of age (Erickson, 1966). This age-dependent exhaustion of the primordial pool of follicles in other species has been reviewed by Byskov (1978) and Richards (1980). The dynamics of antral follicular growth during estrous cycles in rats and mice have been achieved through radiolabeling techniques and morphological analysis (Peters, 1978; Richards, 1980). Since these radiolabeling and morphometric techniques require large sample sizes, measuring the dynamics of growth of antral follicles within an estrous cycle of a cow would be difficult to accomplish. Therefore, measurements such as numbers of various size follicles and mean sizes of various types of follicles have predominantly been used in cattle to assess follicular growth. Numbers of antral follicles within any particular size category or stage of growth are related to: 1) the rate of entry (or progression) of growing, preantral follicles into the pool of antral follicles, 2) the rate of growth of these antral follicles into a larger size category and 3) the rate of loss (atresia) of these follicles from a large-size category into a smaller-size category. If these three rates were constant, then the number of follicles of all sizes would remain constant throughout the estrous cycle. However, there is significant variation in numbers of various size antral follicles during the bovine estrous cycle (Rajakoski, 1960; Marion et al., 1968; Swanson et al., 1972; Schams et al., 1977; Ireland et al., 1979; Matton et al., 1981; Merz et al., 1981; Staigmiller and England, 1982). Therefore, these changes in numbers of follicles within different size categories during an estrous cycle

could result from any combination of these three factors. It is in this perspective that a review of attempts to measure growth of antral follicles in cattle will be presented.

*Relationship between Diameter and Volume.*

Since follicles can be located at various depths in the ovarian stromal tissue, it is unlikely that reliable measurements of the diameter of antral follicles are obtained unless follicles are removed from the ovary and excess stromal tissue removed. Therefore, we examined the relationship between diameter and fluid volume of bovine follicles. Thirty-two pairs of ovaries were collected from cattle between 20 and 30 d after parturition, and follicles were removed from the ovary and dissected free of stromal tissue. Follicular diameter was estimated by measuring length at both long and short axes with a vernier calipers. Volume of follicular fluid was estimated by aspirating the fluid into a graduated syringe. Follicular diameter and fluid volume of 243 follicles were significantly correlated ( $r=.86$ ) with each other (figure 1A). A similar curvilinear relationship was observed between diameter and volume in human follicles (McNatty et al., 1979b). However, logarithmic transformation of both diameter and volume resulted in a significant linear relationship ( $r=.98$ , figure 1B). A significant linear relationship also has been reported for ovine follicles ( $r=.99$ ) when follicular diameter is regressed against the cube root of follicular fluid volume (England et al., 1981; Tsonis et al., 1984). This relationship between diameter and volume can be used to standardize and compare data from one study in which, for example, only follicular diameter was measured to another study in which only follicular volume was measured. We also observed that follicular weight was highly correlated with both diameter ( $r=.89$ ) and volume ( $r=.99$ ).

*Follicular Growth during Repetitive Estrous Cycles.*

Few studies have been conducted with serially sectioned ovaries to characterize ovarian follicular development during the bovine estrous cycle, most likely due to the limitations mentioned previously. The first and most extensive study was published by Rajakoski (1960), who proposed that two "waves" of follicular growth exist during the estrous cycle, d 1 to 12 and d 13 to 0 (d 0 = estrus). However, this hypothesis was based on a non-statistical, quantitative evaluation of across-day trends of follicular numbers in spite of a six-fold difference in the number of follicles  $\geq 5$  mm in

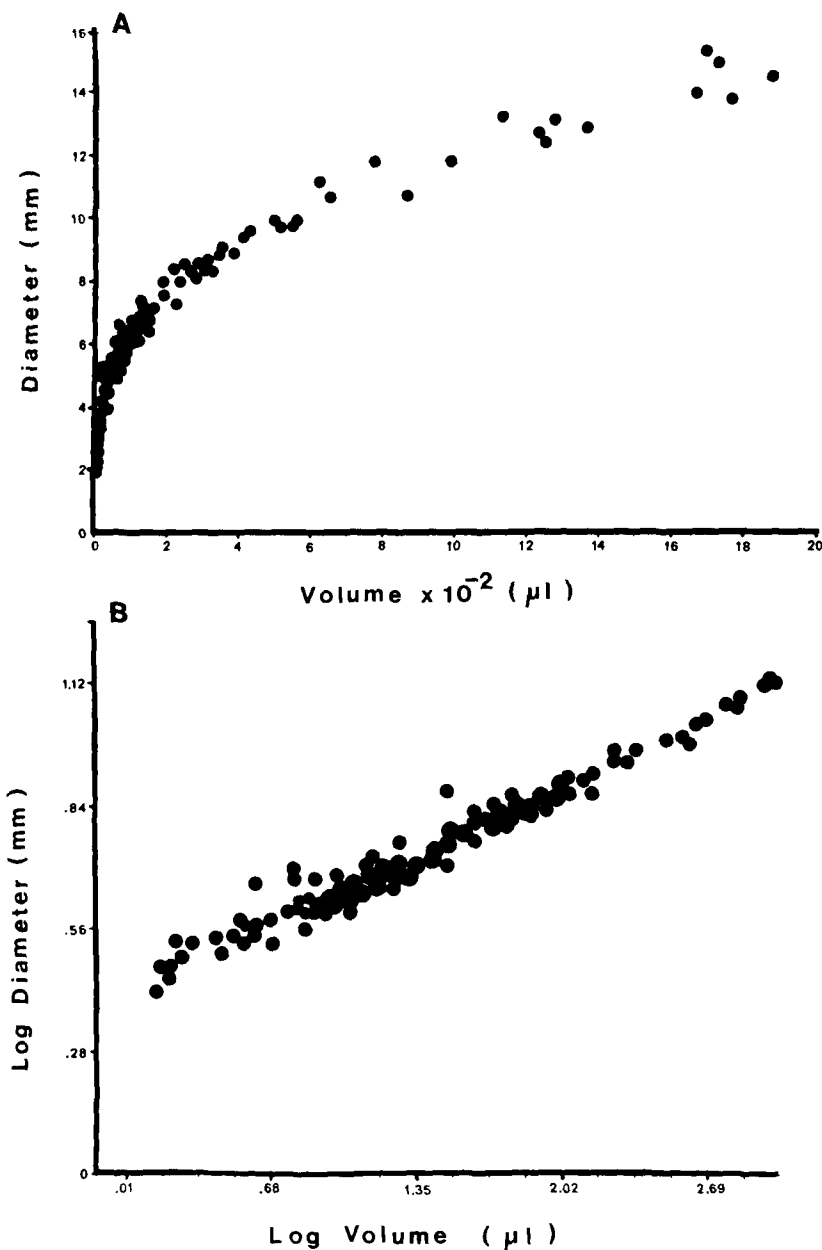


Figure 1. Relationship between follicular diameter and follicular fluid volume of 243 follicles dissected from 32 pairs of ovaries. (A) simple correlation coefficient of actual data,  $r=.868$ . (B) simple correlation coefficient of  $\log_{10}$  vs  $\log_{10}$  plot,  $r=.985$ .  $Y = .266x + .267$ .

diameter that existed among cows within days. This large among-cow variation confounded the across-day trends observed because only one cow per day was inspected on 9 of the 21 d. If one were to allot the data into seven groups, each encompassing a 3-d interval and containing data from four to seven cows, only

one "wave" of large antral follicular growth (numbers) would be detected (figure 2). An increase in the total number of large antral follicles ( $\geq 5$  mm) also has been found to occur from d 1 to 18 of the bovine estrous cycle (Swanson et al., 1972; Schams et al., 1977; Merz et al., 1981), along with a decrease

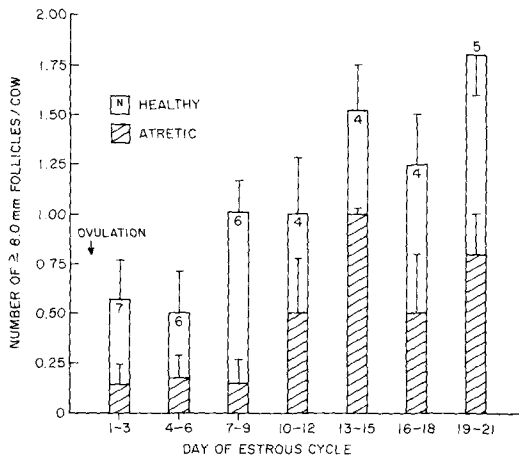


Figure 2. Numbers of healthy and atretic follicles 8.0 mm or greater quantified at various days of the bovine estrous cycle (Data revised from Rajakoski, 1960). N=number of cows.

in number of smaller (1 to 3 mm) antral follicles (Matton et al., 1981). From Rajakoski (1960), it also can be determined that there is an increase in the rate of atresia of large antral follicles between d 7 and 15 (figure 2). A study similar to Rajakoski's has been reported by Kruip (1982). If data from Kruip (1982) were categorized as shown in figure 2, the number of atretic large ( $\geq 8$  mm) follicles per cow changes from .5 on d 1 to 3 to 1.0 on d 19 to 21. This increase in rate of atresia of large antral follicles was also observed by Choudary et al. (1968) and Merz et al. (1981). Therefore, the available evidence suggests that an increase in the rate of growth of small antral follicles into larger antral follicles coincides with an increase in the rate of atresia of large antral follicles as the estrous cycle progresses toward ovulation.

Measuring an increase in size of the one or two largest follicles on each ovary is another way to assess follicular growth. In all (Donaldson and Hansel, 1968; Hackett and Hafs, 1969; England et al., 1973; Matton et al., 1981) but one (Merz et al., 1981) study, it was found that the diameter of the largest ovarian follicle increases between d 1 and 20 of the bovine estrous cycle (d 0 = day of estrus). Matton et al. (1981) indicated that follicular growth and replacement (turnover) of follicles  $>6$  mm diameter are more rapid after d 13 than before d 8. Most large follicles persist on the ovarian

surface for at least 5 d between d 3 and 13 of the estrous cycle. After d 13, most of these large follicles disappear (or turnover) within a 5-d period and are replaced by new, previously smaller follicles (Matton et al., 1981). Moreover, growth of medium-sized follicles (3 to 6 mm diameter) occurs only when the largest follicle is rapidly turning over. In addition, Ireland and Roche (1983b) found that the diameter and volume of large "estrogen-active" follicles (concentration of estradiol  $>$  progesterone in follicular fluid) increases from d 17 through the end of the luteinizing hormone (LH) surge (approximately 1 d before ovulation). Thus, one could suggest from these data that the largest antral follicle present on the ovary enlarges as estrus approaches. However, the probability increases only at d 18 that the largest follicle finally ovulates (Dufour et al., 1972; Matton et al., 1981). The precise time at which an individual follicle enters the growing phase and the number of estrous cycles completed before an individual antral follicle undergoes atresia are unknown in the cow. Based upon a mitotic index of ovine granulosa cells, Scaramuzzi et al. (1980) estimated that about 22 d are required for a bovine follicle to grow from small antral (.4 mm diameter) to large antral size (10.0 mm diameter). Preliminary data presented by Lussier et al. (1983) indicate that two estrous cycles are required for a follicle to grow from pre-antral size to an antral size of 8.5 mm by using a mitotic index of bovine granulosa cells. Other preliminary data suggest that a growing follicle may require about 5 d to be recruited for maturation and to reach its functional maturity at estrus (Fortin and Seguin, 1984). With this evidence, a model for follicular growth can be proposed (figure 3). Each sigmoidal line depicts the path individual follicles follow during growth (solid line) and atresia (dotted line). The slope (growth rate) and height (follicular size) of the growth lines increase as the cycle advances. Thus as the number of both solid and dotted lines increase, follicular turnover increases. It can be hypothesized (figure 3) that the total number (healthy plus atretic) of large follicles (obtained by the number of sigmoidal lines that can be intersected by a given vertical line) increases as time of ovulation approaches. The rate (slope of sigmoidal lines) at which follicles enter, grow, and become atretic accelerates as ovu-

lation approaches. This acceleration does not seem to occur in primates (Hodgen et al., 1985) or rodents (Richards, 1980). The process whereby selection of the follicle that finally ovulates is unknown.

*Follicular Growth during Postpartum Anestrus.* The limited information available on ovarian antral follicular growth in cattle during postpartum anestrus suggests that follicular growth increases markedly after the first week postpartum (Saiduddin et al., 1968b; Wagner and Hansel, 1969; Kesler et al., 1980), and that large antral follicles (>10 mm diameter) may be present within 5 wk prior to the first postpartum estrus (Wiltbank et al., 1964). Thus, large antral follicles are present during postpartum anestrus, but they do not ovulate soon after they appear. Although challenge doses of gonadotropin-releasing hormone (GnRH) or estradiol can induce normal gonadotropin surges by this time (Short et al., 1979; Kesler et al., 1980; see later section), perhaps physiologic increases in estradiol (10 to 15 pg/ml blood) produced by large follicles may be incapable of stimulating preovulatory gonadotropin surges.

Alternatively, these large follicles may be unable to produce sufficient estradiol for appearance of normal estrual rise in estradiol. Results of these studies are based on observations of only the large follicles ( $\geq 5$  mm) present in ovaries; thus, quantitative dynamics of ovarian follicular growth in cattle during postpartum anestrus remain unknown. Recently, Dufour and Roy (1985) found that after microscopic evaluation of ovaries collected from dairy cows, the percentage of the total follicular population per ovary that was nonatretic, small antral follicles (.16 to .28 mm) decreased significantly from d 15 to 25 postpartum, with no change in percentage of atretic follicles in this size category; whereas the percentage of slightly larger, nonatretic follicles (.29 to 1.57 mm) increased during the same interval. Although not significant, there was also a trend for nonatretic antral follicles >1.57 mm to increase 32% from d 15 to 25 postpartum (Dufour and Roy, 1985). These results suggest that there is growth of small antral follicles into larger follicles during this postpartum period in dairy cows. Additional

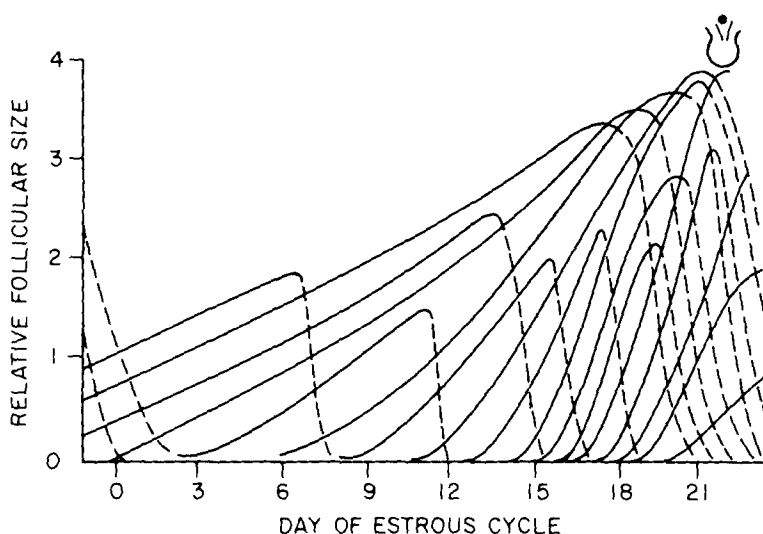


Figure 3. Schematic diagram of large antral follicular growth and atresia during the bovine estrous cycle. The y-axis depicts relative changes in follicular size, and the x-axis depicts day of the estrous cycle. Solid lines represent growth paths of follicles and dotted lines represent atresia paths of follicles. Slopes of the solid lines depict relative rate of follicular growth. The number of sigmoidal lines indicate relative number of follicles.

preliminary data indicate that large follicles ( $\geq 8$  mm) are present on the ovarian surface as early as d 7 postpartum, and that numbers of follicles 4 to 7.9 mm diameter increase with time after parturition in acyclic, suckled beef cows (Spicer et al., 1983). Rate of turnover of large antral follicles during postpartum anestrus is unknown.

#### **Follicular Growth in Cattle as Compared with Other Mammals**

In this section follicular growth is compared among species in order to comment on some potential mechanisms in the cow.

*Primate.* Ovarian folliculogenesis in humans and nonhuman primates is similar to cattle in that usually only one follicle ovulates each reproductive cycle. However, primate cycles (28 d) differ from cattle in that the follicular phase is much longer in primates (14 d) vs cattle (5 d). The majority of studies conducted in primates have utilized rhesus monkeys (diZerega and Hodgen, 1981; Zeleznik, 1982; Hodgen et al., 1985). Hodgen and coworkers describe their data as "recruitment" and "selection" of the "dominant" ovarian follicle. In contrast to cattle, the follicle destined to ovulate in the rhesus monkey is "selected" or chosen about 14 d before ovulation. This selected follicle gradually increases in size and maintains "dominance", or its eminence on the ovary, until it ovulates. Control of this selection process is unclear. Selection of the ovulatory follicle in cattle seems to occur during a brief period ( $\leq 5$  d) and its dominance is uncertain. The initial stimulus for selection of the preovulatory follicle in cattle may involve increased LH pulse frequency observed before preovulatory gonadotropin surges (Rahe et al., 1980; Schallenberger et al., 1985b). The presence of large ovarian follicles in cattle can also influence growth and function of small follicles (Matton et al., 1981; Staigmiller and England, 1982), suggesting some "dominance" by large follicles.

*Sheep.* Ovulation rates in sheep depend on the breed, and range from one to five per ewe. Thus control of folliculogenesis in single-ovulating breeds of sheep may be similar to cattle. Regardless of breed, however, it appears that ewes, like cows, have short ( $\leq 5$  d) follicular phases (for reviews see Cahill, 1984; Driancourt et al., 1985b).

Recent studies in ewes designed to explain

differences in ovulation rates among various breeds suggest several possibilities. Higher ovulation rates may result from: 1) larger secondary preovulatory peaks of follicle-stimulating hormone (FSH; Cahill et al., 1981); 2) a greater number of large antral follicles with lower atretic rates (Lahlou-Kassi and Mariana, 1984); 3) prolonged recruitment and low intensity of selection of follicles destined to ovulate (Driancourt et al., 1985a); or 4) a need for more follicles to produce the same quantity of estradiol to initiate preovulatory gonadotropin surges (McNatty et al., 1985a).

*Swine.* Sows are multiple ovulators, usually ovulating 7 to 14 follicles per cycle. Thus, control of folliculogenesis in sows is most likely very different from cows, even though the length of the estrous cycle is similar (21 d). It seems that more than 5 d are needed for selection of the group of follicles to be ovulated at the ensuing estrus in sows (Clark et al., 1982).

*Rodents.* Similar to pigs, rats are multiple ovulators. However, with only a 4-d reproductive cycle, follicular growth and recruitment are rapid in rats (Peters, 1978; Richards, 1980). Unlike the cow or primate, preovulatory gonadotropin surges in rats recruit (or select) a cohort of follicles that grow coincidentally, and it is this group of follicles that ovulates at the time of the next gonadotropin surges (Richards, 1980).

#### **Ovarian Follicular Steroidogenesis During Repetitive Estrous Cycles**

*Introduction.* The ovaries of cattle are the major source of estrogens, androgens and progesterone found in peripheral blood (Kanchev et al., 1976). Consequently, concentrations of these steroids in peripheral blood may measure follicular and ovarian function. In addition to concentrations of steroids in blood, follicular steroidogenic capability may be ascertained by quantifying concentrations of steroids in fluid. There is strong evidence to indicate a high positive correlation between in vitro follicular cell steroid production and concentration of steroids in follicular fluid (Channing, 1980; Hillier et al., 1981; Bieszczad et al., 1982; Marut et al., 1983). Thus, the next section will describe changes in concentrations of estradiol, androgens and progesterone in blood and follicular fluid during repetitive estrous cycles and then, in a later section,

during postpartum anestrus in cattle. A section also is included addressing a potential role that inhibin and other intrafollicular peptides may have in follicular function.

#### *Steroids in Peripheral Blood*

**Estradiol.** At the time of estrus in cattle, concentrations of estradiol in blood increase two- to six-fold above mid-luteal phase levels (Henricks et al., 1971; Wettemann et al., 1972; Echternkamp and Hansel, 1973; Dobson and Dean, 1974; Chenault et al., 1975; Smith et al., 1975; Bartol et al., 1981; Kotwica and Williams, 1982). Several studies reported that during the luteal phase of the estrous cycle, concentrations of estradiol in blood fluctuate unpredictably (Mason et al., 1972; Wettemann et al., 1972; Echternkamp and Hansel, 1973; Kotwica and Williams, 1982; Schallenberger et al., 1985b). In several other studies, however, a 1.5- to fourfold rise in blood concentrations of estradiol occurred between d 3 and 11 (Henricks et al., 1972; Shemesh et al., 1972; Glencross et al., 1973; Peterson et al., 1975; Smith et al., 1975; Kanchev et al., 1976; Lukazewska and Hansel, 1980), but in only two of these seven studies (Henricks et al., 1972; Glencross et al., 1973) was this rise statistically significant. Perhaps a secondary rise in serum estradiol does not occur in all cows. This possibility is supported by findings of Echternkamp and Hansel (1973) in which only 20% of the cows exhibited two estradiol peaks during the estrous cycle. Alternatively, infrequent sampling of blood may account for some discrepancies in results regarding a secondary estradiol rise, since Kotwica and Williams (1982) found acute, and often marked, changes in concentrations of estradiol when blood samples are collected every 1 to 6 h during the mid-luteal phase. A report (Schallenberger et al. 1985b) in which blood samples were collected every 20 min for 12 h at 3- to 5-d intervals supports the conclusions that estradiol concentrations fluctuate randomly throughout the luteal period.

**Androgens.** The few studies in which androgens in peripheral blood have been measured during the bovine estrous cycle indicate that their concentrations vary markedly throughout the cycle in an unpredictable pattern (Shemesh and Hansel, 1974; Kanchev et al., 1976; Kanchev and Dobson, 1976; Kotwica and Williams,

1982; Wise et al., 1982b). In some of these studies (Shemesh and Hansel, 1974; Kanchev and Dobson, 1976; Kotwica and Williams, 1982), increases in concentrations of peripheral testosterone and estradiol occurred concurrently. Since androgens are precursors of estrogens, changes in concentrations of androgens in serum may reflect periods of changing follicular function during the estrous cycle. However, during certain times of the cycle, the aromatase system in the bovine follicle has the capacity to utilize more androgen than is endogenously available, since exogenous testosterone can cause marked increases in ovarian estradiol secretion and hasten the onset of preovulatory gonadotropin surges (Kotwica and Williams, 1982).

**Progesterone.** Concentrations of progesterone are greatest (2 to 10 ng/ml) during d 8 to 18 of the estrous cycle (Wettemann et al., 1972; Glencross et al., 1973; Kanchev et al., 1976; Schams et al., 1977; Kotwica and Williams, 1982). Between d 20 and 3, when the corpus luteum has regressed, concentrations of progesterone in peripheral blood are low (<1 ng/ml; Wettemann et al., 1972; Glencross et al., 1973; Kanchev et al., 1976; Schams et al., 1977; Spicer et al., 1981; Kotwica and Williams, 1982). These results indicate that the corpus luteum is the primary source of progesterone, and therefore, any change in follicular secretion of progesterone during the luteal phase likely will be overshadowed.

#### *Steroids in Follicular Fluid*

**Estradiol.** Lunaas (1964) was first to observe that total ovarian estradiol concentrations in cattle were nearly 10-fold greater during the preovulatory period than any other day of the estrous cycle. In comparison, fluid from several follicles pooled into three size categories contained concentrations of estrogen that were highly variable within a given stage of the estrous cycle (Ireland et al., 1979). In addition, individual follicles collected on various days of the estrous cycle have highly variable concentrations of estradiol in follicular fluid (with at least one follicle capable of producing high amounts of estradiol during most days of the cycle; Bartol et al., 1981; Merz et al., 1981; Ireland and Roche, 1983a,b). Perhaps this highly variable production of estradiol accounts for the inconsistent pattern of concentrations of estradiol in blood previously reported during

d 1 to 17 of the cycle. In contrast to a major part of the estrous cycle, there appear to be consistent short-term changes in concentrations of estradiol in follicular fluid during the pre-ovulatory period. Concentrations of estradiol in fluid of large follicles ( $\geq 6$  mm) are high or increase slightly during the 3 to 5 d before the gonadotropin surges, and then decline after the LH surge (Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982; Dieleman et al., 1983a,b; McNatty et al., 1984a,c; Fortune and Hansel, 1985) coincident with changes in concentrations of estradiol in peripheral serum and utero-ovarian venous serum (Ireland et al., 1984; Fogwell et al., 1985). Estradiol produced by bovine follicles originates primarily from granulosa cells and not thecal cells (McNatty et al., 1984b). In general, size of antral follicles in bovine ovaries is positively correlated with fluid estradiol concentrations, whereas size is negatively correlated with androstenedione concentrations (Ireland et al., 1979; Kruip et al., 1979; Henderson and Franchimont, 1981; Ireland and Roche, 1982).

*Androgens.* Little emphasis has been placed on the temporal pattern of follicular fluid concentrations of androgens during the estrous cycle, even though androgens are present in bovine follicles in nanogram per milliliter concentrations (Short, 1962) and serve as precursors for follicular estrogen production (Richards, 1980; Hillier, 1981). In bovine follicles, thecal cells are the major source of follicular androstenedione (McNatty et al., 1984b). It was also found that concentrations of androgens in follicular fluid were measurable but did not change in large follicles ( $\geq 6$  mm) collected at 2-d intervals from heifers between d 3 and 13 (Ireland and Roche, 1983a), indicating that the low follicular estradiol secretion observed during most of the estrous cycle is not due to a lack of an aromatizable precursor. Additional evidence indicates that large atretic bovine follicles ( $\geq 8$  mm) have significantly lower LH-stimulated androstenedione production in vitro than healthy follicles (Henderson et al., 1984c; McNatty et al., 1985b). Concentrations of androgens in fluid of large antral follicles increased (Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982; Dieleman et al., 1983a,b; Fortune and Hansel, 1985) just before and during the LH surge, but decreased after the LH surge. This decrease in androgen levels was coincident with the decline in concentrations of

estradiol and the increase in concentrations of progesterone in follicular fluid of these same follicles. Whether the decrease in estradiol observed after the LH surge is due to the reduction in aromatizable androgen and(or) a result of a shift in the steroidogenic pathway toward luteinization remains to be determined.

*Progesterone.* The presence of large quantities of progesterone in bovine follicular fluid has been well established (Short 1962; Ireland et al., 1979). It is also well known that intra-follicular progesterone serves as a precursor to androgen and, subsequently, estrogen production by follicles. Both granulosa and thecal cells of bovine follicles produce large amounts of progesterone (McNatty et al., 1984a).

The steroidogenic capability of individual bovine follicles to produce progesterone at various stages of the estrous cycle, as measured by follicular fluid concentrations, appears to increase only near the time of ovulation (Ireland and Roche, 1982, 1983a,b; Staigmiller and England, 1982; Dieleman et al., 1983a; Fortune and Hansel, 1985). Perhaps this increase in intrafollicular progesterone is a result of preovulatory luteinization of granulosa cells, as observed in other species (Channing, 1980), or inhibition of aromatization (Dieleman and Blankenstein, 1984). Certainly, the decline in intrafollicular concentrations of estradiol seen prior to ovulation (Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982; Dieleman et al., 1983a) is not a result of declining production of progesterone.

Atresia presumably is characterized by impaired estrogen production and abnormal cytology of granulosa cells. Thus, estrogen to progesterone ratios have been used to categorize bovine follicles as healthy or atretic. High estrogen to progesterone ratios are associated with healthy bovine follicles that contain low numbers of picnotic nuclei in granulosa cells, and low estrogen to progesterone ratios are associated with atretic follicles that contain numerous granulosa cells with picnotic nuclei (Ireland and Roche, 1982, 1983a,b; Bellin and Ax, 1984; Bellin et al., 1984). However, actual correlation coefficients between morphological atresia and estrogen to progesterone ratios, although significant, are low ( $r=.18$ , Bellin and Ax, 1984). This suggests that several other variables are involved in the process of atresia in addition to follicular fluid levels of estrogen and progesterone. Other intrafollicular correlates to atresia in bovine



follicles include chondroitin sulfate (Bellin and Ax, 1984; Bushmeyer et al., 1985), albumin and lactate dehydrogenase (Wise, 1985).

**Follicular Fluid Inhibin.** Recently, Padmanabhan et al. (1984) observed an increase in inhibin-like activity in "estrogen-active" follicles approximately 24 h before prostaglandin  $F_{2\alpha}$ -induced preovulatory gonadotropin surges. During the increase in follicular fluid inhibin, LH in serum increased whereas FSH secretion was unchanged (Padmanabhan et al., 1984). Inhibin-like activity then decreased to low levels through the gonadotropin surges. Associations between follicular fluid inhibin-like activity and serum FSH have also been observed in rats (Fuji et al., 1983). Padmanabhan et al. (1984) hypothesized that increased inhibin production by preovulatory (estrogen-active) follicles prevents an increase in FSH secretion and thus, recruitment of additional ovulatory follicles. This hypothesis is supported by observations that ewes immunized against inhibin, partially purified from bovine follicular fluid, had higher ovulation rates than control ewes (Henderson et al., 1984b; O'Shea et al., 1984). However, in vitro findings indicate inhibin can exert marked interactions with estradiol and progesterone in control of gonadotropin secretion (Massicotte et al., 1984), but increased inhibin secretion may not be the sole determinant in altering gonadotropin secretion during the preovulatory period. Evidence suggests that follicular fluid inhibin originates from the granulosa, since granulosa cells collected from rats (Sander et al., 1984) and cows (Henderson and Franchimont, 1981) produce inhibin-like activity in vitro. In addition, differences in the ability of bovine granulosa cells to produce inhibin-like activity in vitro exist between atretic states of follicles (Henderson et al., 1984a). However, more work is required to develop an immunoassay for inhibin now that bovine inhibin has been isolated (Robertson et al., 1985), and to determine how changes in follicular numbers and health contribute to the level of inhibin that is secreted by the ovary in vivo.

Other untested possibilities to explain selection of one ovulatory follicle in cattle in addition to differential inhibin production (Padmanabhan et al., 1984) include: differential production of FSH-binding inhibitor (Darga and Reichert, 1979; Sluss and Reichert, 1984), differential production of a more recently identified aromatase inhibitor (di-

Zerega et al., 1982), differential production of insulin-like growth factors (Adashi et al., 1985) and/or simply, differential production of estradiol as hypothesized in monkeys (Zelevnik et al., 1984).

#### Ovarian Follicular Steroidogenesis during Postpartum Anestrus

##### *Steroids in Peripheral Blood*

**Estradiol.** In cattle, concentrations of estradiol in peripheral blood decrease sharply at parturition to basal levels (1 to 8 pg/ml) within 2 to 6 d (Henricks et al., 1972; Echternkamp and Hansel, 1973; Arije et al., 1974; Sasser et al., 1979; Humphrey et al., 1983), and then increase just before the first postpartum estrus (Henricks et al., 1972; Echternkamp and Hansel, 1973; Arije et al., 1974; Corah et al., 1974; Rawlings et al., 1980; Williams and Ray, 1980; Chang et al., 1981; Humphrey et al., 1983). This increase in preovulatory estradiol is similar in duration and magnitude to that observed during repetitive estrous cycles. However, first postpartum ovulations that occur without estrus often are followed by a short luteal phase (Wettemann, 1980). It is unknown if increases in blood estradiol are "normal" before these short luteal phases. Whether ovarian follicles have the ability to secrete estrogen well before the first postpartum ovulation is also unknown.

**Androgens.** Studies reporting androgens in peripheral blood during the postpartum anestrus period were not identified.

**Progesterone.** Concentrations of progesterone in serum are low (<1.0 ng/ml) at parturition due to the preparturient regression of the corpus luteum of pregnancy. Concentrations of progesterone remain low in cows until initiation of estrous cycles (Robertson, 1972; Rawlings et al., 1980; Wettemann, 1980; Humphrey et al., 1983). In 40 to 70% of cows examined, a small progesterone peak (<2 ng/ml) occurs 1 to 6 d before the first postpartum estrus (Robertson, 1972; Rawlings et al., 1980; Wettemann, 1980; Humphrey et al., 1983). This increase in concentration of progesterone in peripheral blood which precedes the first postpartum estrus, may result from formation of a transitory corpus luteum (Donaldson et al., 1970) or luteinization of some follicles (Berardinelli et al., 1979). However, these structures are unable to maintain normal luteal phase progesterone secretion (Kesler et

al., 1981). The cause of the shortened life span of these corpora lutea (or luteinized follicles) is unknown, but may involve excess prostaglandin production by the uterus (Troxel and Kesler, 1984a; Troxel et al., 1984).

#### *Steroids in Follicular Fluid*

Few data are available on steroid concentrations in follicular fluid collected during postpartum anestrus. In one study, no difference in in vitro production of estradiol, progesterone, testosterone or androstenedione by antral follicles of various sizes (1 to 15 mm) was found between d 25 to 30 postcalving and d 10 to 16 of the estrous cycle (Weiss et al., 1981). In another study (Walters et al., 1982a), total ovarian follicular fluid content of estradiol and progesterone on d 25 after parturition was not different between nonsuckled and suckled cows, even though the nonsuckled cows were approaching first estrus (Walters et al., 1982c). In contrast, Bellin et al., (1984) found that on d 5 postpartum, cows with suckling calves had significantly lower estradiol concentrations in follicles  $\geq 3$  mm diameter than those in which the calves were weaned within 24 h after birth. Because there is large variation between concentrations of steroids in follicular fluid of individual follicles within a cow (Merz et al., 1981; Bellin and Ax, 1984), it is not surprising that differences in follicular fluid steroid levels were not observed by Weiss et al. (1981) or Walters et al. (1982a), who obtained data from pooled follicles. Thus, at present, insufficient data are available to determine how and when follicular steroidogenesis changes during the postpartum period. Obtaining this information is important because functional capabilities of ovaries may be limiting re-establishment of estrous cycles. Preliminary evidence suggests that in large ( $\geq 8$  mm) follicles of acyclic cows, follicular fluid concentrations of first progesterone, then estradiol, increase with time postpartum (Spicer et al., 1983, 1984b). However, there is no change in the steroidogenic capacity of smaller follicles with time postpartum (Spicer et al., 1984b). This suggests that large follicles may need to obtain the capacity to produce estradiol prior to first ovulation postpartum. Thus, circumstances shortly after parturition may allow follicular development to a large size (see "Follicular Growth during Postpartum Anestrus"), but not the development of enzyme systems required

for adequate production of estrogen. Recent evidence also indicates that the level of nutrition during the postpartum period may play a role in follicular estradiol production (Rone et al., 1983).

#### **Gonadotropins in Peripheral Blood**

Ovarian function is associated with concentrations of gonadotropins in blood. Thus, this section of the review will describe the changes in concentrations of both LH and FSH that occur in blood of cattle during the postpartum interval. Changes in gonadotropin secretion during the bovine estrous cycle have been reviewed elsewhere (Hansel and Convey, 1983). Therefore, only analogies will be drawn between preovulatory events during repetitive estrous cycles and the first postpartum ovulation.

#### *Luteinizing Hormone*

**Dairy Cattle.** In dairy cattle that are milked twice daily (calves removed at parturition), peripheral blood levels of LH increase during the first 1 to 3 wk postpartum (Erb et al., 1971; Echtenkamp and Hansel, 1973; Ingalls et al., 1973; Kesler et al., 1977; Fernandes et al., 1978; Goodale et al., 1978; Schallenberger et al., 1978; Webb et al., 1980; Peters et al., 1978; Bolt and Rollins, 1983) and remain elevated during the postpartum anovulatory period (Erb et al., 1971; Fernandes et al., 1978; Schallenberger et al., 1978). This increase in overall concentrations of LH in peripheral blood is probably due to increased frequency of pulsatile LH release from  $<1$  pulse per 6 h to 1 or 2 pulses per 6 h (Carruthers and Hafs, 1980; Peters et al., 1981; Little et al., 1982). Increased pulsatile release of LH may be due to an acceleration of pulsatile release of LH-releasing hormone (LHRH) from the hypothalamus (Schallenberger and Peterson, 1982). Increases in pulsatile release of LH in serum also occur before the preovulatory LH surge in cyclic cattle (Rahe et al., 1980; Schallenberger et al., 1985b). However, these preovulatory changes in frequency of pulsatile LH release are more dramatic, increasing from 1 or 2 pulses of LH per 6 h during mid-luteal phase to 3 to 8 pulses per 6 h during late follicular phase.

During the postpartum anovulatory period, LH concentrations after LHRH treatment are greater after d 14 postpartum than during d 3

to 10 (Kesler et al., 1977; Fernandes et al., 1978; Schallenberger et al., 1978; Azzazi et al., 1983). Thus, this increased ability of the pituitary to secrete LH in dairy cows occurs well before the first postpartum ovulation (Wettermann, 1980).

**Beef Cattle.** In suckled beef cows, concentrations of LH in peripheral blood do not increase to maximal levels until after the third or fourth week postpartum (Peters et al., 1981; Williams et al., 1982; Convey et al., 1983; Humphrey et al., 1983). Frequencies of LH pulses during postpartum anestrus range from 1.2 to 2.2 pulses per 6 h in beef cattle (Walters et al., 1982b; Convey et al., 1983; Humphrey et al., 1983). It is uncertain whether increased frequency and/or amplitude of LH pulses contribute to increased concentrations of LH in blood, since both frequency (Humphrey et al., 1983) and amplitude (Convey et al., 1983) increase with time postpartum. Increased pulsatile release of LHRH may be involved in the increase in LH pulses (Walters et al., 1982b; Convey et al., 1983; Humphrey et al., 1983). The low frequency and/or amplitude of LH pulses during late gestation in cattle (Little et al., 1982; Schallenberger et al., 1985a) may be due to high progesterone and estrogen concentrations in peripheral blood (Goodman and Karsch, 1980), and with parturition, the hypothalamus and/or pituitary gland may recover from negative feedback effects of these steroids, resulting in an increase in LH pulses (Lamming et al., 1982).

Concentrations of LH in blood after LHRH treatment of beef cows are greater 20 d after parturition than during d 3 to 20 (Webb et al., 1977; Williams et al., 1982; Gauthier and Mauleon, 1983). As with dairy cows, it appears that this increase in the ability of the anterior pituitary gland to secrete LH in beef cows occurs well before the first spontaneous postpartum ovulation (Wettermann, 1980).

#### *Follicle Stimulating Hormone*

In the few studies reported, concentrations of FSH in peripheral blood do not appear to change in dairy or beef cattle during the first 14 to 50 d postpartum (Schallenberger et al., 1978; Williams et al., 1982; Bolt and Rollins, 1983; Convey et al., 1983). In addition, FSH concentrations after LHRH treatment are greater 10 d after parturition than during d 3 to 5 in both beef and dairy cows (Schallenberger et al., 1978; Williams et al., 1982), suggesting that normal secretory activity of

FSH returns in cows earlier than that of LH. These and other results suggest that the ability of the pituitary gland to secrete gonadotropins in cattle per se may not be the limiting factor for return to cyclic ovarian activity after the second week postpartum (Kesler et al., 1977; Leung et al., 1983; Moss et al., 1985).

Hypothalamo-pituitary-ovarian interactions during the bovine estrous cycle are reviewed elsewhere (Hansel and Convey, 1983). Briefly, two main factors are needed to elicit pre-ovulatory gonadotropin surges: 1) a decrease in concentrations of progesterone in blood and 2) an increase in concentrations of estradiol in blood. As mentioned earlier, steroid changes may also be involved in changing the pulsatile secretion of LH after parturition, as well as during the interval before the gonadotropin surges. Apparently, increased basal secretion of LH observed 2 or 3 d before the gonadotropin surges is due to increased frequency of LH pulses (Rahe et al., 1980; Schallenberger et al., 1985b), and occurs without a change in basal secretion of FSH (Padmanabhan et al., 1984; Schallenberger et al., 1985b). Frequency of FSH pulses increases only slightly during the preovulatory period, from 3 or 4 pulses per 6 h during the mid-luteal phase to 5 or 6 pulses per 6 h during late follicular phase (Schallenberger et al., 1985b). How an increase in pulsatile secretion of LH alters follicular steroidogenesis remains unclear.

#### **Effects of Exogenous Steroids on Postpartum Intervals**

**Dairy Cows.** Since small, transient increases in serum progesterone are often observed before normal postpartum estrous cycles, exogenous progesterone treatments have been given in attempts to hasten time to first ovulation. However, in some studies interval to first postpartum ovulation in dairy cows is prolonged with progesterone treatment (Fosgate et al., 1962), or unaltered (Britt et al., 1972, 1974; Azzazi et al., 1983), whereas conception rates are unaltered (Fosgate et al., 1962; Britt et al., 1972) or improved (Britt et al., 1974). Dose, frequency and time progesterone was injected postpartum and nutritional status of the dam (Dunn and Kaltenbach, 1980) may add to variation in response to progesterone.

Although suckling vs milking prolongs the interval from calving to first estimated ovulation in intact cows (Edgerton, 1980) and in unilaterally ovariectomized, estradiol-treated dairy cows (Stevenson et al., 1983), estradiol

treatment, when combined with 100 µg LHRH, does not alter days to first estimated ovulation in milked dairy cows (Azzazi et al., 1983). However, responsiveness of the hypothalamo-pituitary axis to large doses of estradiol increases with time postpartum (Azzazi et al., 1983; Stevenson et al., 1983), thus indicating that postpartum increases in serum estradiol need to be timed properly to elicit normal gonadotropin surges.

*Beef Cows.* Progesterone or Synchro-Mate-B pretreatment before human chorionic gonadotropin (hCG) injection (Sheffel et al., 1982), LHRH challenge (Smith et al., 1984; Troxel and Kesler, 1984b) and early weaning (Ramirez-Godinez et al., 1981) reduces the incidence of short luteal phases in postpartum beef cows but does not appear to improve reproductive performance as measured by pregnancy rate (Smith et al., 1979). The mechanism by which progestin pretreatment increases the capacity of follicles to ovulate and form functional corpora lutea in response to endogenous or exogenous gonadotropins is unknown.

Various combinations of progestins and(or) estrogens without hCG, LHRH or weaning to induce ovulation and estrus have reduced the interval to first postpartum ovulation and estrus in beef cows (Foote and Hunter, 1964; Saiduddin et al., 1968a; Brown et al., 1972; Wettemann, 1980; Williams et al., 1980), whereas interval to conception is either reduced (Foote and Hunter, 1964) or unaltered (Saiduddin et al., 1968a; Brown et al., 1972). Again, effectiveness of such steroid treatments may be influenced by numerous variables such as dose and injection regimens of steroids, nutritional status of dams, and suckling intensity of calves on dams (e.g., one vs two calves).

As in dairy cows, estradiol can induce LH secretion and(or) ovulation in suckled beef cows (Short et al., 1979; Irvin et al., 1981; Echternkamp and Spicer, 1984; Peters, 1984). This estradiol-induced LH release is dependent on the dose of estradiol, time after parturition estradiol is given, and suckling intensity (Radford et al., 1978; Short et al., 1979; Schallenberger et al., 1982; Peters, 1984). It requires about 2 or 3 wk for the hypothalamo-pituitary axis to recover and fully respond to positive feedback effects of estradiol (Short et al., 1979; Peters, 1984). However, at the same time after parturition, low doses of estradiol do not

stimulate much LH release, whereas large doses of estradiol cause LH surges (Short et al., 1979). Thus, physiologic increases of estradiol in peripheral blood (10 to 15 pg/ml) during the first few weeks postpartum may not be able to stimulate normal preovulatory gonadotropin surges. This suppressed LH release may be due to increased sensitivity of the hypothalamus and(or) pituitary to the negative feedback of estrogen during this postpartum period in suckled cows, as suggested by Acosta et al. (1983). It is unclear what proportion of estradiol-induced postpartum ovulations results in normal luteal development.

#### Ovarian Responsiveness to Exogenous Gonadotropins After Parturition

Although estradiol secretion (Echternkamp, 1978; Wettemann et al., 1982) and ovulation (Casida et al., 1943; Foote et al., 1966; Oxenreider, 1968; Echternkamp, 1978; Rovira et al., 1978) can be induced with exogenous gonadotropins in lactating beef cows prior to 45 d postpartum, it appears that the gonadotropin-induced corpus luteum has a shorter lifespan in as high as 75% of the animals (Foote et al., 1966; Echternkamp, 1978; Wettemann et al., 1982; Troxel et al., 1983). Echternkamp (1978) also observed minimal increases in plasma estradiol concentrations to the gonadotropic stimulus in some cows. Thus, follicular growth or differentiation in many postpartum cows may be at a point unsuitable for subsequent normal luteal development, even though follicles are responsive to gonadotropins. Perhaps these follicles lacked their potential maturity (i.e., did not have a complete complement of gonadotropin receptors and(or) follicular cells). This may also be the case for many first postpartum ovulations, since it was found that corpora lutea removed after the first postpartum ovulation were smaller and contained fewer live cells than those obtained after subsequent ovulations (Duby et al., 1985).

Interval to first postpartum ovulations, whether induced or spontaneous, is influenced by several other factors including nutritional status of the dam (Dunn and Kaltenbach, 1980; Williams et al., 1980; Hansen et al., 1982; Bartle et al., 1984; Rutter and Randel, 1984), and season and stress (Christenson, 1980; Hansen and Hauser, 1983; Lewis et al., 1984). Thus, care should be taken when interpreting results of several different experiments.

**Binding of Gonadotropins to  
Ovarian Follicles during  
Repetitive Estrous Cycles**

The concept that the responsiveness of follicles depends not only on changes in concentrations of gonadotropins in serum but also on changes in the concentration of hormone-binding sites (or receptors) in cellular membranes of follicles has gained considerable attention during the past few years. Changes in follicular function may be associated with changes in numbers of follicular gonadotropin-binding sites. Thus, this section of the review will describe changes in the cellular concentrations of ovarian LH- and FSH-binding sites, first during repetitive estrous cycles, and secondly, during postpartum anestrus. Since little is known about receptors for gonadotropins in follicles in cattle or sheep, research in other mammals will be included.

**Luteinizing Hormone Receptors.** Between diestrus and proestrus, the concentrations of LH receptors in granulosa cells increase in pigs (Stouffer et al., 1976) and rats (Cheng, 1976; Uilenbroek and Richards, 1979). Similarly, the concentration of LH receptors in granulosa cells from large follicles increases dramatically between onset of the preovulatory LH surge and peak of the LH surge, and subsequently declines just prior to ovulation in sheep (Webb and England, 1982a,b) and cattle (Ireland and Roche, 1982, 1983b; Staigmiller and England, 1982; Staigmiller et al., 1982; Walters et al., 1982c). These results suggest that final preovulatory maturation of ovarian follicles is associated with increased numbers of LH receptors in granulosa cells and, hence, increased sensitivity of granulosa cells to concentrations of LH in blood.

The concentration of LH receptors in granulosa cells removed from large follicles ( $\geq 6$  mm) is low on d 3, 5, 11 and 13 of the estrous cycle in heifers (Ireland and Roche, 1983a). On d 7, however, concentrations of LH receptors in granulosa cells are two- and fourfold higher than any of the other days measured. Ireland and Roche (1983a) speculated that this rise in numbers of LH receptors on d 7 is indicative of development of potential ovulatory follicles that subsequently undergo atresia after d 7.

Numbers of LH receptors in thecal tissue appear to follow similar changes, as observed in the granulosa cells of the same follicles in rats (Uilenbroek and Richards, 1979), ewes (Webb and England, 1982a,b) and heifers (Ireland and Roche, 1982, 1983a,b; Staigmiller et al., 1982).

**Follicle Stimulating Hormone Receptors.** Cheng (1976) found that levels of FSH receptors (binding sites) in rat ovarian homogenates increased nearly fourfold between diestrus and proestrus, only to decrease between proestrus and estrus, and again decrease between metestrus and diestrus. However, the binding data in this study were expressed on a tissue-weight basis and may not reflect changes per cell. Indeed, when granulosa cells were isolated from rat ovaries and binding data expressed per microgram of DNA, no change in concentrations of FSH receptors was noted throughout the rat estrous cycle (Uilenbroek and Richards, 1979).

In cattle, concentrations of FSH receptors in granulosa cells removed from large follicles during the preovulatory period (Ireland and Roche, 1982, 1983b; Staigmiller et al., 1982; Walters et al., 1982c) and from d 3 to 13 of the estrous cycle (Ireland and Roche, 1983a) did not change. These results suggest that final preovulatory maturation of ovarian follicles is not associated with a change in concentrations of FSH receptors (and hence, sensitivity to FSH) in granulosa cells.

Compared with granulosa cells, thecal tissue concentrations of FSH receptors are low (Carson et al., 1979; Richards and Kersey, 1979; Uilenbroek and Richards, 1979). This low binding of FSH to thecal tissue probably reflects contamination of the thecal component with granulosa cells (Carson et al., 1979; Richards, 1980).

**Binding of Gonadotropins to  
Ovarian Follicles during  
Postpartum Anestrus**

**Gonadotropin Receptors.** To our knowledge, there have been no longitudinally designed studies that characterize changes of either LH or FSH receptors in ovarian follicles during the postpartum anestrus period of any mammalian species. Walters et al. (1982a,b) found that numbers of LH receptors in pooled follicular homogenates were significantly higher on d 25 after parturition in nonsuckled than suckled cows. Since nonsuckled cows were approaching first estrus (Walters et al., 1982c) this may indicate that receptors for LH increase in follicles prior to first postpartum ovulation. This increase in numbers of LH receptors also is observed near ovulation in cyclic heifers (Ireland and Roche, 1982; Staigmiller and England, 1982; Staigmiller et al., 1982; Walters et al., 1982c). Number of follicular FSH receptors did

not differ between nonsuckled and suckled cows (Walters et al., 1982c). Similarly, there is no change in number of FSH receptors during the estrous cycle of cattle (Ireland and Roche, 1982, 1983a,b). Preliminary evidence suggests that in anestrous cows injected every 2 h with LHRH (500 ng), the largest follicle responds by 96 h with an increase in number of both thecal and granulosa LH binding sites with no change in number of FSH binding sites (Spicer et al., 1984a). This increase in LH-binding sites coincided with an increase in concentrations of estradiol in follicular fluid at 96 h.

#### Prepubertal Follicular Development

Numbers of antral follicles in prepubertal heifers increase significantly from birth to 3 mo of age (Erickson, 1966; Goff et al., 1984). However, between 3 and 9 mo of age, numbers of antral follicles remain constant (Howe et al., 1964; Erickson, 1966; Forrest et al., 1984) until the onset of puberty (or first estrus; Desjardins and Hafs, 1969; Swanson et al., 1972). Results from a preliminary study suggest that, although ovarian follicles collected from heifers at 3, 6 and 9 mo of age contain low but measurable amounts of estradiol (Forrest et al., 1984), concentrations in follicular fluid do not differ among these ages. Nonetheless, the sensitivity of the hypothalamo-pituitary axis to positive feedback control of estradiol increases during this prepubertal period (Swanson and McCarthy, 1978; Staigmiller et al., 1979), as it also does during the postpartum period. Negative feedback control exerted by estradiol also exists during this time in heifers, but evidence suggests that the sensitivity remains constant and only decreases just before the onset of puberty (Kiser et al., 1981; Schillo et al., 1982; Day et al., 1984; Moseley et al., 1984). Studies assessing changes in negative feedback effects of estradiol during postpartum anestrus should also be conducted. Although heifers 1 to 6 mo old respond to exogenous gonadotropins with an increased number of large antral follicles and ovulation (Casida et al., 1943; Howe et al., 1964; Jainudeen et al., 1966; Onuma et al., 1970; Seidel et al., 1971), the response is variable. The time ovarian follicles obtain their full complement of gonadotropin receptors and(or) steroidogenic enzymes in order to consistently and fully respond to gonadotropic stimulus before puberty and after parturition is unknown in cattle.

#### Ovarian Blood Flow and Follicular Function

Since the ovary is an endocrine gland, changes in blood flow may be part of the overall response of the gland to stimulation. However, studies on this regulatory aspect of ovarian follicular function have been limited. The reader is referred to more detailed and comparative reviews on ovarian blood flow (Ginther, 1974; Niswender et al., 1976; Ellinwood et al., 1978; Ford, 1982; Levasseur, 1983). The majority of reports concentrate on hormonal regulation of uterine blood flow. A detailed account of changes in ovarian function and blood flow during repetitive estrous cycles in cattle has recently been published (Wise et al., 1982a). Changes in ovarian blood flow during the reproductive cycle in sheep (Nett et al., 1976; Niswender et al., 1976; Reynolds et al., 1984), pigs (Magness et al., 1983; Reynolds and Ford, 1984) and monkeys (Wehrenberg et al., 1977) have also been reported. Generally, blood flow to the ovary is highest during the luteal phase and lowest during the follicular phase. In addition, increases in concentrations of progesterone in peripheral blood are significantly correlated with increases in ovarian blood flow. This is in contrast to the uterus, where the higher the estrogen to progesterone ratio in systemic blood, the greater is the quantity of blood flowing through the uterine vascular bed (Ford, 1982; Ford et al., 1984). Other factors suggested to be regulators of uterine blood flow include prostaglandins, catecholamines (e.g., epinephrine) and catecholestrogens (Ford, 1982). Few mechanisms for control of ovarian blood flow have been suggested in the literature. Little is known about the innervation of the ovarian vasculature, but changing vasculature innervation is one potential site of regulation (Bahr et al., 1974).

Reports on other aspects of ovarian blood flow include countercurrent-concentrating mechanisms for providing additional androgen substrate for follicular metabolism and(or) aromatization in sows (Krzymoski et al., 1982a,b) and cows (Kotwica et al., 1982). However, the role these and other mechanisms play in follicular growth and steroidogenesis is unclear. Some questions that remain are: How would an increase in blood flow stimulate steroidogenesis? Could an increase in blood flow remove inhibitors of steroidogenesis or enhance the countercurrent concentrating

mechanisms? Could estradiol or other steroids alter the ability of the ovarian vascular bed to respond to nerve stimulation as postulated for the uterus (Ford, 1982)? Can an alteration in blood flow occur differentially within a single ovary and direct the blood supply from one follicle to another as postulated for the dominant follicle in primates (Zelevnik et al., 1981). Finally, vasoconstrictors such as prostaglandins and epinephrine both stimulate granulosa cell steroidogenesis in vitro (Hsueh et al., 1984), but in vivo are such substances also acting by some indirect mechanism on the vasculature?

#### Follicular Steroidogenesis and Oocyte Maturation

One goal of preovulatory follicular development is to provide an oocyte capable of maturation and fertilization once ovulated from the follicle. Thus, a brief summary of the relationship between follicular growth, steroidogenesis and oocyte maturation is appropriate. Detailed reviews on control of oocyte maturation (Thibault, 1977; Tsafiriri, 1978; Wassarman et al., 1979; Channing et al., 1982; Ball et al., 1984) in mammals are available. Besides in vivo preovulatory maturation of the oocyte, maturation of oocytes in vitro, as measured by resumption of meiosis, can be accomplished by removal of the oocyte from inhibitory effects of follicular cells. These inhibitors may be steroidal or nonsteroidal (Hammond, 1981; Channing et al., 1982), and the role of various substances in the maturation of mammalian oocytes may vary with species (Crosby et al., 1985).

In vitro maturation of bovine oocytes is not influenced by follicular diameter (Thibault et al., 1976; Leibfried and First, 1979; Fukui and Sakuma, 1980; Leibfried-Rutledge et al., 1985), and is probably not dependent on direct action of gonadotropins (Fukui et al., 1982). Addition of estradiol and progesterone has improved the proportion of bovine oocytes maturing in vitro in one study (Fukui et al., 1982), whereas these steroids had no effect in another study (Foote and Thibault, 1969). Studies in humans suggest that exposure of oocytes to high levels of both estradiol and progesterone before ovulation may stimulate maturational competence (McNatty et al., 1979a; Channing et al., 1983). Recently, high follicular-fluid estradiol levels correlated well with successful fertilization and enhanced

cleavage rate of human oocytes (Botero-Ruiz et al., 1984; Hillier et al., 1984). Bovine oocytes collected from follicles of superovulated cattle also can be fertilized in vitro (Bondioli and Wright, 1983). However, more studies are needed to define relationships among levels of follicular fluid steroids, oocyte maturation and fertilization in cattle. For example, within a given follicular size category, do fluid steroid levels correlate with oocyte maturation and/or fertilization? Preliminary data suggest that in bovine follicles 5 mm and greater, high progesterone and low estradiol concentrations in follicular fluid are associated with the ability of oocytes to mature in vitro (Grimes and Ireland, 1984; Grimes et al., 1985).

#### Conclusions

After re-evaluation of data published by Rajakoski (1960), coupled with results of other studies, we hypothesize that final preovulatory follicular growth <5 d before ovulation is reflected by increased rate of atresia of large antral follicles. Concurrent with this increased atresia are increased rates of growth of small antral follicles into large follicles and increased turnover (replacement) of large follicles.

Rising titers of estradiol in peripheral blood during the preovulatory period may be due to increased production of estradiol by large follicles which, in turn, may be due to increased responsiveness (i.e., increase in LH receptors) to increased pulses of LH in blood. Inconsistent changes in estradiol production have been observed during the luteal phase of the cycle and may reflect random follicular growth and steroidogenesis.

The mechanism for selection of the follicle destined to ovulate is unclear. Increased pulses of LH observed during the preovulatory period may likely play a role. Specific proteins (e.g., inhibin, FSH-binding inhibitor, aromatase inhibitor) produced by the "selected" preovulatory follicle may aid in its assurance to ovulate. One cannot rule out potential interactions these intrafollicular peptides have with steroids on ovarian follicles and the anterior pituitary gland. Substances that regulate ovarian blood flow also may be involved in follicular selection.

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